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Research Article/Araştırma Makalesi

Hybridisation of Turkish isolates of *Cordyceps militaris*: cultivation method and biological-efficiency analysis

Cordyceps militaris (L.) Fr. Türk izolatlarının melezlenmesi: yetiştirme teknikleri ve biyolojik etkinlik analizi

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ABSTRACT

Cordyceps militaris is a filamentous entomopathogenic fungus prized for its spectrum of antiviral metabolites and its promise as an eco-friendly mycoinsecticide, yet these qualities vary markedly among genotypes. To assess the breeding potential of Turkish germplasm, fruiting bodies were collected from Ümraniye City Forest (İstanbul); single ascospores were isolated, and the fastest-growing, morphologically vigorous colonies were retained. Ten hybrids were generated by pairing isolates with complementary mating-type genes (MAT1-1 × MAT1-2), propagated in liquid culture, and evaluated on rice substrate. Biological efficiency (BE = [fresh stroma ÷ dry substrate × 100]) ranged from 0% to 37.5%; one hybrid failed to fruit, whereas Y08 × Y33 achieved a near-commercial 37.5% biological efficiency. Although these local hybrids currently trail top commercial strains, the dataset highlights substantial heritable variation and suggests that incorporating simple gene-editing upgrades could elevate well-adapted Turkish lines to ≥ 50% efficiency without sacrificing regional ecological fitness. The study therefore underscores the commercialisation potential of indigenous C. *militaris* and provides a foundation for future strain-improvement and bioproduct development programmes

Key Words: Biological efficiency, *Cordyceps militaris*, cultivation, entomopathogenic fungus, hybridization

ÖZ

Geniş bir antiviral metabolit yelpazesi ayrıca çevre dostu bir mantar bazlı insektisit olma potansiyeli nedeniyle değerli görülen *Cordyceps militaris*, hiflerden oluşan bir entomopatojenik mantardır. Ancak bu özellikler genotipler arasında belirgin şekilde değişiklik göstermektedir. Türk genetik materyalinin ıslah potansiyelini değerlendirmek amacıyla mantarlar Ümraniye Kent Ormanı'ndan (İstanbul) toplanmış; tek askosporlar izole edilmiş ve en hızlı büyüyen, morfolojik olarak en canlı koloniler korunmuştur. Tamamlayıcı eşleşme tipi genlerine (MAT1-1 × MAT1-2) sahip izolatların eşleştirilmesiyle on hibrit üretilmiş, sıvı kültürde çoğaltılmış ve pirinç substratında değerlendirilmiştir. Biyolojik etkinlik (BE = [taze stroma ÷ kuru substrat × 100]) %0 ile %37,5 arasında değişmiş; bir hibrit meyve vermemişken, Y08 × Y33 hibriti ticari seviyeye yakın %37,5 biyolojik etkinlik elde etmiştir. Bu yerel hibritler mevcut en iyi ticari hatların gerisinde kalsa da, veri seti önemli kalıtsal varyasyonu ortaya koymakta ve basit gen düzenleme geliştirmelerinin bölgesel ekolojik uyumu koruyarak iyi uyarlanmış Türk hatlarını ≥ %50 etkinliğe yükseltebileceğini düşündürmektedir. Bu çalışma dolayısıyla yerli C. *militaris*'in ticarileştirilme potansiyelini vurgulamakta ve gelecekteki hat geliştirme ve biyopestisit geliştirme programları için bir temel sunmaktadır.

Anahtar Kelimeler: Biyolojik etkinlik, *Cordyceps militaris*, entomopatojenik mantar, hibritleşme, kültüre alma

Introduction

Cordyceps militaris (L.) Fr. (*Hypocreales: Cordycipitaceae*) is a well-documented entomopathogen of Lepidoptera. Taxonomically it belongs to the phylum Ascomycota and is morphologically recognised by its bright-orange, club-shaped stromata bearing embedded perithecial ascomata (Sung et al., 2007). Infection is initiated when conidia or ascospores adhere to the larval or pupal cuticle, germinate, form appressoria and enzymatically breach the integument; once inside the haemocoel the fungus proliferates, kills the host and finally produces external stromata that forcibly discharge ascospores for onward dissemination (Kato et al., 2024). Although highly insect-specific in nature, *C. militaris* can be cultivated saprophytically on grain or liquid substrates, so it is not a strict obligate parasite (Wu et al., 2021). Under temperate field conditions it usually completes a single generation per year that is synchronised with host metamorphosis. Documented field epizootics on pine-processionary moth *Thaumetopoea pityocampa* (Denis & Schiffermüller, 1775) together with laboratory assays showing ≥ 90% mortality in early-instar *Helicoverpa zea* (Boddie, 1850) at similar doses (Glover et al., 2023) illustrate both its natural impact and its biocontrol potential.

Cordyceps militaris has matured into a dual-purpose “green factory,” prized for both its high-value secondary metabolites and its entomopathogenic capacity. Complementing conventional breeding, CRISPR/Cas9 edits have been used to adjust the genes that steer cordycepin synthesis in laboratory yeasts, nudging production to roughly 3 g L^{-1} without altering the organism’s core biology (Tan et al., 2023; Song et al., 2023). The same metabolite suite reinforces potent insecticidal activity: foliar applications quickly kill early-instar *H. zea*, and cadaver sporulation enables secondary cycling in the field (Glover et al., 2023). Field surveys in Lithuanian Scots-pine stands have likewise recovered *C. militaris* from up to 59% of overwintering *Dendrolimus pini* (Linnaeus, 1758) larvae, highlighting the fungus’s ecological compatibility with coniferous forest pests (Gedminas et al., 2015). Because it also grows well in both submerged and solid-state fermentation, *C. militaris* is increasingly viewed as a scalable chassis for concurrent production of high-value metabolites and environmentally benign biocontrol agents (Hu et al., 2024; Peng et al., 2024).

Effectiveness as a mycoinsecticide depends on close genetic matching between fungal strain and agroecosystem. Vietnamese isolates, for example, caused 70–100% mortality in *Galleria mellonella* (Linnaeus, 1758), whereas Lithuanian isolates infected only 4–59% of *D. pini* (Gedminas et al., 2015; Nguyen et al., 2022). Routine DNA barcoding (ITS, TEF-1α) and MAT1-1/MAT1-2-guided hybridisation can therefore improve both field performance and metabolite output; a recent cross doubled cordycepin and carotenoid levels without sacrificing sporulation (Lin et al., 2021), and earlier balanced crosses increased stroma biomass five-fold (Zhang and Liang, 2013). Subsequent genome editing allows fine-tuning to local needs (Hu et al., 2024), and extension specialists are now advocating region-adapted formulations for integrated pest management (Saranya and John, 2021).

This study evaluates whether MAT-guided hybridisation of 79 DNA-barcoded Turkish *C. militaris* isolates can produce high-yield, genetically stable strains suited to mass production and field use. Ten carefully selected MAT1-1/MAT1-2 crosses were evaluated in a uniform three-stage cultivation system, generating the first quantitative performance dataset for *C. militaris* in Türkiye and addressing a gap highlighted by recent pathogenicity surveys (Nguyen et al., 2022). Top-performing hybrids—combining robust sporulation with elevated cordycepin, carotenoid, and stroma yields—provide a fast-track towards region-adapted mycoinsecticides against major lepidopteran pests such as *H. zea* (Glover et al.,

2023) and establish a breeding template for future strain-improvement efforts.

Material and Methods

Field collection and morphological identification

Approximately 50 morphologically intact fruiting bodies of *Cordyceps militaris*—representing independent field isolates—were collected on 17 December 2024 from a single site in Ümraniye City Forest, İstanbul, Türkiye (41.0649–41.0656 °N, 29.1051–29.1094 °E). In the laboratory, each specimen was confirmed as *C. militaris* based on the diagnostic characters described by Liu et al. (2011). Each stroma originated from an insect pupa buried immediately beneath the soil surface; according to the diagnostic evaluation of Trematerra and Colacci (2018), these pupae were identified as belonging to the pine processionary moth (*Thaumetopoea pityocampa*). To maintain aerobic respiration while minimising external contamination, each specimen was individually wrapped in breathable cellulose tissue and transported to the Sakarya Mushroom Agrifood Industry & Trade Ltd. R&D Laboratory within 2 h of collection. Representative field specimens are shown in Figure 1.

Figure 1. Field-collected *Cordyceps militaris* fruiting bodies and an intact stroma–pupa complex.

Ascospore isolation

The ascocarp (stroma) of *Cordyceps militaris* bears numerous small, flask-shaped perithecia. The inner walls of these perithecia are lined with slender, cylindrical asci (sing. ascus); after one meiotic division followed by mitosis, each ascus typically encloses eight ascospores—a diagnostic "8-spored asci" pattern characteristic of the genus *Cordyceps*. Collected specimens were identified to species under bright-field illumination at 400 × magnification using the morphological criteria of Shrestha et al. (2025).

Ascospore discharge ("firing") in *C. militaris* occurs via forcible ejection from the perithecia, a hallmark of Ascomycota driven by turgor pressure within the ascus (Trail, 2007) (Figure 2). From the collection, the ten healthiest stromata—free of tissue decay or disease and exhibiting well-developed perithecia—were selected for ascospore isolation.

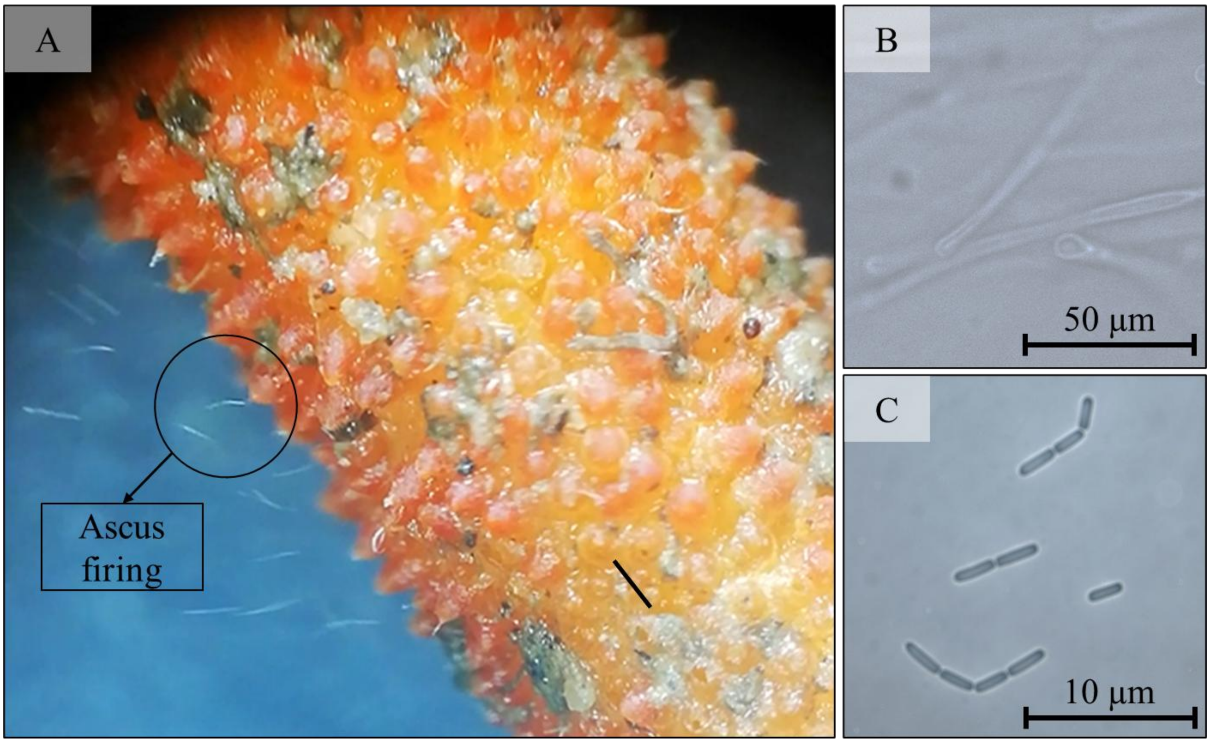


Figure 2. Micro-morphology of *Cordyceps militaris*: (A) asci discharging from a mature perithecium; (B) cylindrical asci at 400 ×; (C) liberated ascospores at 400 ×.

Each stroma was inverted, gently wrapped in cellulose paper, and suspended within an autoclavable glass tube (2.5 cm diameter) so that ascospores could settle at the tube base. The tubes were left undisturbed for two days to allow spore deposition (Figure 3).

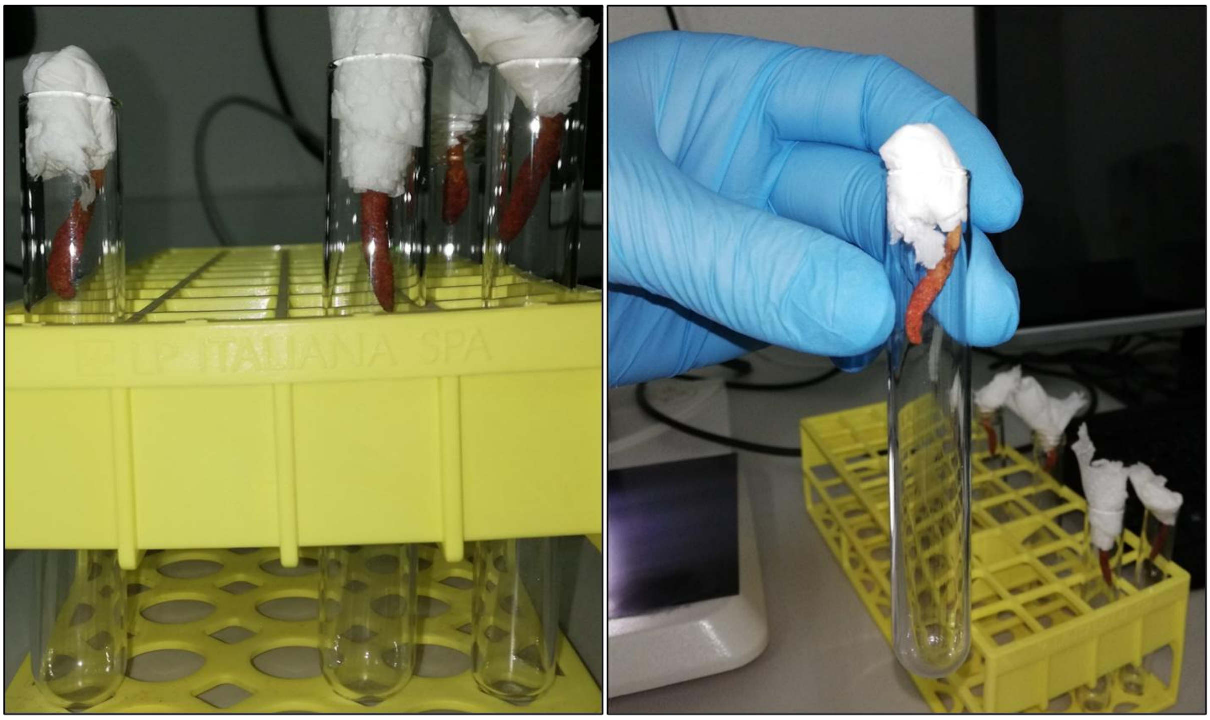


Figure 3. Ascospore collection setup with inverted stromata in 2.5 cm glass tubes.

Colony development and mating-type screening of ascospore isolates

After two days, a distinct layer of ascospores had settled at the bottom of each tube. The stromata were aseptically removed and discarded, and the spores were rinsed into a sterile 500 mL beaker with autoclaved distilled water to a final volume of roughly 100 mL. To dislodge any remaining asci and release adherent spores, the suspension was gently agitated with a

1 × 15 mm Teflon-coated magnetic stir bar at 250 rpm for 15 s. The resulting stock was then serially diluted with sterile distilled water to a working concentration of approximately 1×10^1 ascospores mL^{-1}, suitable for single-ascospore isolation. For germination tests, 200 water–agar plates (2% agar, pH 5.8 – 6.2) were inoculated in a laminar-flow cabinet with 1 mL of the diluted suspension per plate. After incubation at 25 °C, 79 colonies displaying the most rapid, symmetric, and aggressive radial growth were selected and coded Y1 – Y79 (Shrestha et al. 2012**)**.

Each isolate was sub-cultured onto a nutrient-rich medium (per litre: malt extract 20 g, glucose 5 g, yeast extract 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g; pH 5.8 – 6.2) and incubated at 25 °C for 7 d. Colonies exhibiting strong, homogeneous mycelial growth without visible sectoring were retained for mating tests (Shrestha et al. 2006).

Because *Cordyceps militaris* is a heterothallic, bipolar ascomycete, each single ascospore carries either the MAT1-1 (+) or MAT1-2 (–) idiomorph (Vu et al. 2024). Pairwise combinations of randomly chosen isolates (Y1–Y79) were co-cultivated on the same agar plate to identify compatible partners. Where hyphae of opposite mating type fused (plasmogamy), a conspicuous dense interaction zone—commonly termed a mating line—developed (Nolting and Pöggeler 2006; Li et al. 2023). The ten pairings that produced the most pronounced mating lines were retained as heterokaryotic (dikaryotic) strains. Mycelial plugs from each heterokaryon were transferred to standard fruiting substrates for subsequent comparison of stroma yield (Figure 4).

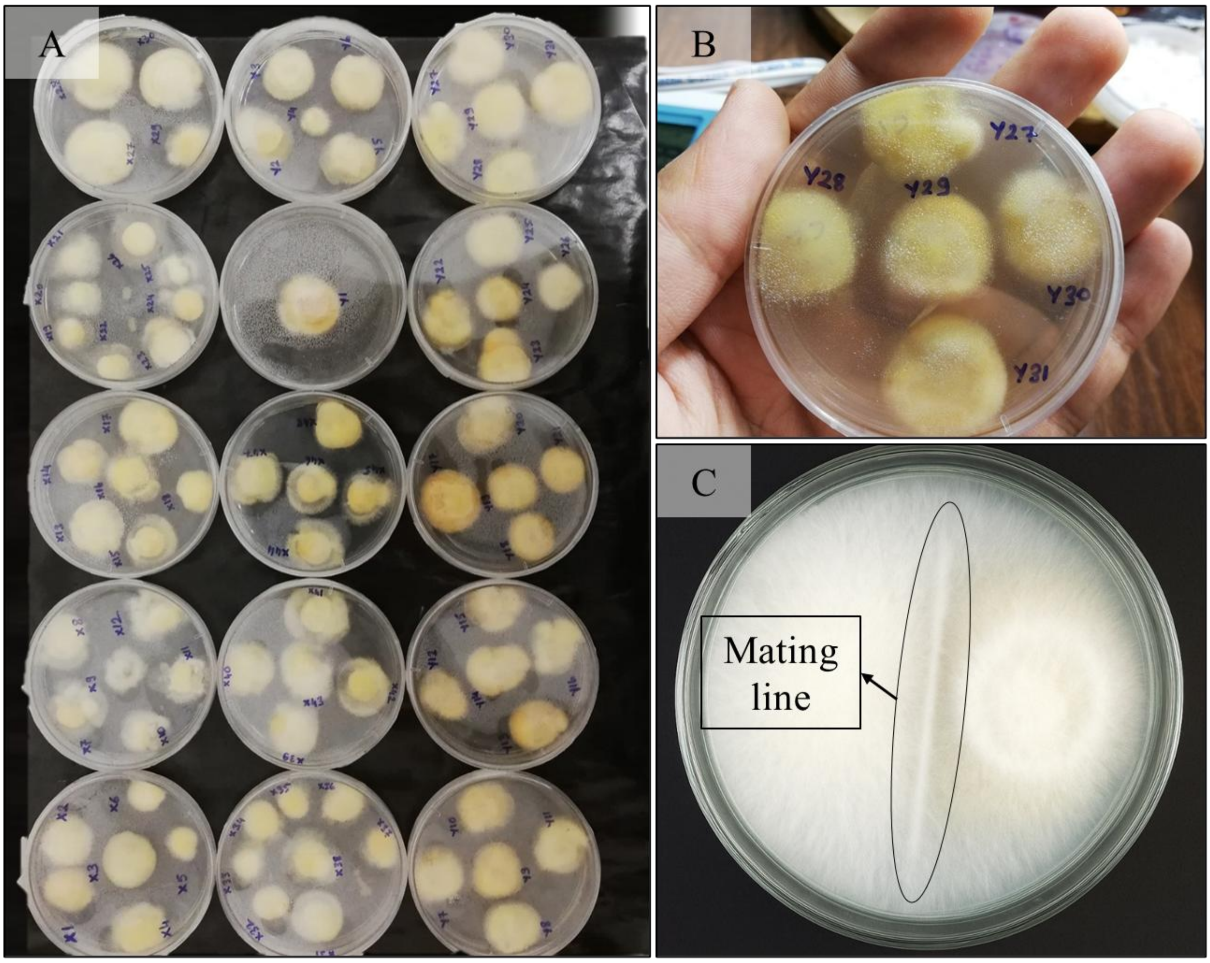


Figure 4. Single-ascospore colonies of *Cordyceps militaris* (A), the subset showing the most aggressive radial growth (B), and the dense mating line formed when compatible isolates are paired (C).

Experimental layout and cultivation cycle of cordyceps militaris

The ten heterokaryotic strains that generated the most distinct mating lines were designated Y28 × Y03, Y08 × Y33, Y28 × Y29, Y27 × Y31, Y28 × Y30, Y31 × Y27, Y15 × Y07, Y25 × Y42, Y09 × Y69, and Y17 × Y55.

For pre-culture, 150 mL of liquid medium (per litre: malt extract 20 g, yeast extract 5 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g; pH 5.8–6.2) was dispensed into 500 mL Erlenmeyer flasks, plugged with cellulose, wrapped in aluminium foil, and autoclaved at 121 °C for 15 min (2 atm). After cooling, each flask was inoculated with the corresponding heterokaryotic strain and incubated on a rotary shaker at 150 rpm and 25 °C for 7 days, conditions widely used for *Cordyceps militaris* seed cultures (Li et al. 2020).

The fully colonised broths were then used to inoculate a rice-based solid substrate (composition in Table 1; final wet weight ≈ 105 g per jar). Jars were fitted with perforated lids covered by autoclavable breathable tape, sterilised under the same conditions, cooled, and aseptically inoculated with 5 mL of the corresponding liquid seed culture. Inoculated jars were incubated under fruit-body-inducing conditions for stroma production and subsequent biological efficiency (BE) comparisons among the hybrid strains (Shrestha et al. 2005).

Each heterokaryotic strain that showed a pronounced mating line—Y28 × Y03, Y08 × Y33, Y28 × Y29, Y27 × Y31, Y28 × Y30, Y31 × Y27, Y15 × Y07, Y25 × Y42, Y09 × Y69, and Y17 × Y55—was inoculated into ten sterile 500 mL jars containing the rice substrate described in Table 1 (wet weight ≈ 105 g; dry weight ≈ 43 g). This yielded 100 jars per run, and the trial was repeated three times (completely randomised design; 300 units total).

Table 1. Composition of culture media used at successive stages of *Cordyceps militaris* cultivation.

Cultivation step (medium)	*Rice*	*Agar*	*Malt extract*	*Yeast extract*	*KH_2PO_4*	*$MgSO_4$*
Ascospore germination (water–agar)	-	20 g L^{-1}	-	-	-	-
Colony selection & pairing (mycelium-run agar)	-	20 g L^{-1}	20 g L^{-1}	5 g L^{-1}	1 g L^{-1}	0.5 g L^{-1}
Liquid seed culture (inoculum)	-	-	20 g L^{-1}	5 g L^{-1}	1 g L^{-1}	0.5 g L^{-1}
Fruit-body substrate (rice solid) per jar*	40 g	-	1.95 g	0.5 g	0.065 g	0.0325 g
** Amounts are per jar containing 65 mL distilled water (final wet weight ≈ 105 g).*						

Jars were inoculated aseptically in a laminar-flow hood, sealed with breathable tape, and incubated at 25 °C in darkness until full colonisation (~21 d). They were then moved to fruiting conditions (18 ± 1 °C, 80–85% RH, 12 h light: 12 h dark) for 40 ± 5 d, giving an overall production cycle of ≈ 60 d (Li et al. 2023) (Figure 5). At harvest the fresh stromata were weighed, and biological efficiency (BE) was calculated for each jar as (Royse 1985)

$$\mathrm{BE} = \frac{fresh\ stroma\ weight\ (g)}{dry\ substrate\ weight\ (g)} \times 100$$

Figure 5. (A) Autoclaved rice substrate, (B) colonised liquid seed culture, (C) aseptic liquid inoculation of the solid substrate, (D) onset and (E) completion of mycelial colonisation, and (F) stroma formation under fruiting conditions.

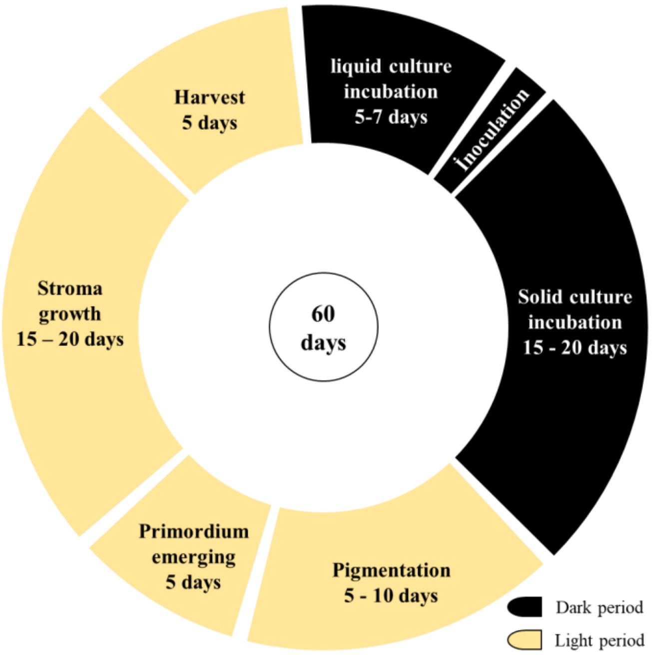
Mean BE values for the ten hybrid strains will be compared by one-way ANOVA followed by Tukey's HSD test (α = 0.05).

The cultivation procedure was divided into three sequential stages—spawn run, primordium induction, and stroma development—each requiring distinct environmental parameters to guide *Cordyceps militaris* from substrate colonisation to mature fruit-body formation.

Stage I – Mycelial colonisation (spawn run): A sterile solid substrate of brown rice or rice supplemented with silkworm-pupa powder (2–4%, w/w) was incubated in darkness at 18 ± 1 °C. Jar lids equipped with PTFE filters (pore size ≥ 0.2 µm) allowed CO_2 exchange while limiting microbial contamination (Hung et al., 2009). Keeping the substrate slightly acidic (pH 5.8–6.2) promotes both radial mycelial growth and cordycepin biosynthesis (Rózsa and Apahidean, 2022). Under a water activity ≥ 0.96 and a C:N ratio of ≈ 20:1, full surface colonisation was achieved within 12–18 d; once the mycelium formed a uniform dull-white mat, the culture was transferred to primordium-induction conditions (Hung et al., 2009).

Stage II – Primordium induction (pigmentation phase): Colonised jars were shifted to a day/night regime of 18 °C / 13–15 °C (ΔT ≈ 4 °C). Relative humidity was held at 90–95%, and the room was ventilated at 2–4 air changes h^{-1} to maintain CO_2 below 1 000 ppm (Wiengmoon et al., 2019). Illumination was provided by white LEDs at 600–1 000 lux for 12–16 h d^{-1}; intensities under 400 lux delayed the carotenoid-based "pinking" response (Wu et al., 2016). Under these conditions the mycelium acquired a pink hue within 4–7 d, and uniform primordia formed within 6–10 d (Wiengmoon et al., 2019).

Stage III – Stroma development (fruiting phase): When primordia became visible, the environmental parameters were adjusted to 22–24 °C by day and 20 ± 1 °C by night, with the relative humidity stabilised at 90–93%. White-LED illumination was maintained at 800–1 500 lux for ≥ 12 h day^{-1}; continuous light of ≥ 1 000 lux has been shown to improve both biological efficiency and cordycepin content (Wu et al., 2016). Carbon-dioxide levels were kept below 800 ppm, and ultrasonic humidifiers were used instead of surface spraying to minimise moisture loss (Wiengmoon et al., 2019). Under these conditions, stromata attained 30–50 mm in length within 10–14 days; harvesting just before apical swelling yielded optimal colour and bioactive-compound levels (Wu et al., 2016) (Figure 6).



Stage I – Spawn-run: 18 ± 1 °C, dark; brown rice ± 2–4 % silkworm-pupa meal; pH 5.8–6.2; a_w ≥ 0.96; C:N ≈ 20:1; full colonisation in 12–18 d.

Stage II – Primordium induction: 18 °C day / 13–15 °C night; RH 90–95 %; CO_2 < 1000 ppm; 600–1000 lux white LED, 12–16 h photoperiod; pinking in 4–7 d, primordia in 6–10 d.

Stage III – Fruiting: 22–24 °C day / 20 °C night; RH 90–93 %; 800–1500 lux, ≥ 12 h light; CO_2 < 800 ppm; stromata reach 30–50 mm in 10–14 d; harvest before apical swelling for optimal colour and cordycepin.

Figure 6. Three-stage cultivation protocol for *Cordyceps militaris*.

Statistical analysis of biological efficiency

All statistical procedures were conducted in Python 3.11 (Python Software Foundation), an open-source programming language, using the Visual Studio Code integrated development environment. Numerical calculations were handled with the NumPy library (v 1.26), data manipulation with pandas (v 2.2), and inferential tests—including one-way ANOVA followed by Tukey’s HSD—with SciPy (v 1.12). Summary graphs were prepared in Microsoft Excel 365. Minor code debugging was aided by the OpenAI ChatGPT language model.

Results and Discussion

One-way ANOVA showed that biological efficiency (BE) differed significantly among the ten *C. militaris* hybrids (F (9, 90) = 34.91, $p < 0.0001$). The corresponding effect size was large ($\eta^2 = 0.78$), indicating that approximately 78% of the variance in BE was attributable to genetic differences between strains (Sung et al. 2006) (Figure 7).

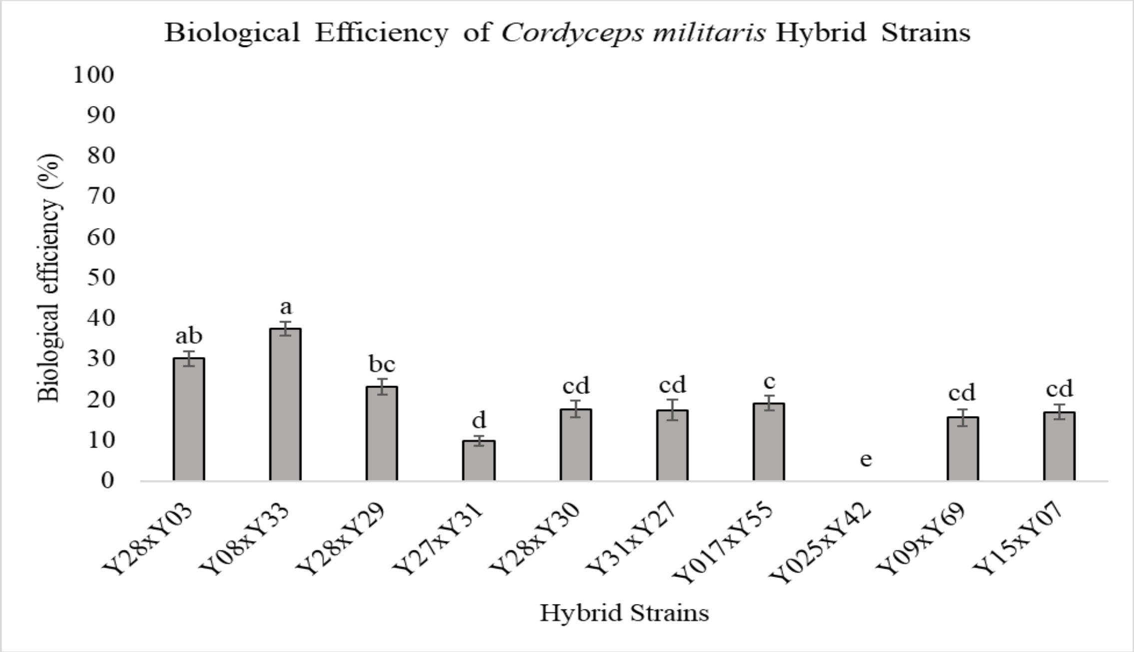


Figure 7. Biological efficiency of ten *Cordyceps militaris* hybrids. One-way ANOVA: $F(9, 90) = 34.91$, $p < 0.0001$, $\eta^2 = 0.78$. Columns sharing the same letter do not differ significantly (Tukey HSD, $\alpha = 0.05$).

Post-hoc Tukey comparisons revealed two statistically superior performers. Hybrid Y08 × Y33 achieved the highest mean BE (37.54%), exceeding every other strain except Y28 × Y03, whose BE averaged 30.14% and did not differ significantly from the top performer. A second tier was represented by Y28 × Y29 (23.22%), which out-yielded the mid-range group—Y28 × Y30, Y31 × Y27, Y17 × Y55, Y09 × Y69, and Y15 × Y07—yet fell short of the two leading strains. Hybrid Y27 × Y31 produced a markedly lower mean BE (9.86%) than all but one genotype. At the opposite extreme, Y25 × Y42 failed to form stromata, resulting in a BE of 0% and constituting a distinct statistical class. In summary, only two of the ten hybrids surpassed the practical benchmark of 30% BE, whereas one genotype failed entirely to fruit. Representative stromata produced by the nine fruiting hybrids are illustrated in Figure 8.

Figure 8. Stromata obtained from each fruiting hybrid on day 60 of cultivation (hybrid Y25 × Y42 excluded; no stroma formed).

Relative to the main benchmarks in the literature, our best Turkish hybrid Y08 × Y33 (37.5% biological efficiency, BE) falls just below the 48–72 % reported for a Chinese MAT-selected cross *(parents carry complementary mating-type genes, MAT1-1 and MAT1-2, that ensure sexual compatibility)* cultivated on rice plus insect-pupa meal (Lin et al., 2021). It nonetheless sits well above the ≈20–28% BE usually recorded for first-generation hybrids obtained by crossing unrelated wild strains and grown on plain rice (Shrestha et al., 2012). Robust and sustained stromatal development is widely regarded as a proxy for genetic integrity: cultures that continue to fruit reliably after successive sub-cultures generally retain full entomopathogenic potency and high titres of hallmark metabolites and digestive enzymes, whereas lines that lose the ability to form fruit bodies (“degenerated” strains) show concurrent declines in both virulence and metabolite production (Yin et al., 2017; Wellham et al., 2021).

Hybrid Y25 × Y42 colonised the rice substrate quickly and evenly—signalling vigorous vegetative growth—yet it never produced fruit bodies (stromata). Because all ten hybrids were grown on the same medium and under identical temperature, humidity and light, an environmental or nutritional cause is highly unlikely; the problem almost certainly lies in the strain’s genetics. Seeing a clear mating line on agar shows that the two parental nuclei recognise each other and begin the sexual pathway, but it does *not* guarantee that fruit bodies will form. Hidden imbalances—such as an extreme excess of one mating type or silent defects in key development genes—can still stall the process after the mycelial phase, leaving a “blankmycelium” that never fruits. Similar cases have been reported in *Cordyceps militaris* when matingtype ratios are strongly skewed or other genetic mismatches are present (Shrestha et al., 2012; Vu et al., 2023; Li et al., 2024).

Recent studies employing the CRISPR gene-editing method demonstrate that just one or two precisely targeted edits can raise biological efficiency (BE) almost as much as multi-generation breeding programmes. For example, deleting a single gene that builds the spore-coat surface elevated BE from ≈50% to 70% without compromising sporulation or insect-killing power (Li et al., 2023), while removing a small toxin-biosynthesis locus eliminated that metabolite yet kept BE steady at ≈45% (Liu et al., 2024). These results point to a practical route for region-adapted strains worldwide: first screen for the highest-performing hybrids, then apply one or two judicious CRISPR edits. Extrapolating the range observed in the present work (0–37.5% BE) to the literature gains suggests that such a “select-then-edit” pipeline could lift elite local strains into the 50–60% BE bracket—comparable to today’s commercial stocks while preserving the ecological fit needed for robust field performance.

Accurate identification and targeted breeding of local Turkish *C. militaris* strains are indispensable because virulence and metabolite titres differ sharply among genotypes (Shrestha et al., 2012; Nguyen et al., 2022). Surveys show that climate-matched isolates can achieve two- to six-fold faster kill (lower LT_{50}) than exotic strains (Kryukov et al., 2018) and may naturally colonise up to 60% of outbreak populations (Gedminas et al., 2015). Likewise, antiviral metabolite levels differ three- to five-fold among strains (Dong et al., 2020), directly affecting both biocontrol potency and biomass value. Our study provides a reference set of barcoded, performance-ranked Turkish hybrids that can now be fine-tuned for both traits.

Conclusion

This study provides the first quantitative map of BE for Turkish *C. militaris* germplasm and identifies Y08 × Y33 as a promising lead line. Although its 37.5% BE trails elite Asian crosses, integrating recurrent selection with simple gene edits could raise local strains to ≥50% BE while maintaining ecological adaptation. Such dual optimisation supports region-specific mycoinsecticides and higher-margin metabolite production from the same cultivation platform, which could enable the large-scale deployment of region-adapted *C. militaris* biocontrol formulations.

Author Contributions

All authors contributed equally to this work.

Study Limitations

This study did not include molecular barcoding (e.g., ITS or TEF-1α sequencing) for each hybrid because of budget limitations, and quantitative profiling of key metabolites such as cordycepin and carotenoids could not be carried out. Future work that combines DNA diagnostics with metabolite analytics will be necessary to pinpoint the genetic drivers of biological-efficiency variation.

Declaration of Competing Interest

The authors have no financial or personal relationships that could inappropriately influence the work reported in this manuscript.

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Data-Availability Statement

The datasets generated and analysed during the present study are available from the corresponding author on reasonable request. No legal, ethical or contractual restrictions apply to their distribution.

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